Microbial Populations in Spacecraft Assembly Facilities Determined by an ATP-based Assay.
K. Venkateswaran[1], N. Hattori[2], S. Chung[1], C. Echeverria[1], W. Schubert[1], G. Kazarians[1], R. Kern[1], T. Sakakibara[2], S. Murakami[2] and C. Basic[1]. [1]Jet Propulsion Lab., Calif. Inst. Tech., Pasadena, USA. [2]Kikkoman Corp., Noda City, Chiba, Japan

Extraterrestrial sample return procedures stress the importance of avoiding contamination of critical hardware components with terrestrial organisms, their remains, or organic matter in general. In order to minimize such contamination of sensitive spacecraft components, the spacecraft clean room environments should remain as free of microbial contamination as possible. A firefly luciferase assay that differentiates free extra-cellular (dead cells, etc.) from intra-cellular (viable microbes) ATP was used to determine the microbial cleanliness of JPL spacecraft assembly rooms. The ATP from viable microbes is determined after destroying free ATP enzymatically, and the cultivable microbial populations enumerated on a solid nutrient rich TSA medium at 32°C. About 650 samples were taken from floors, tables, benches, and other places in clean rooms at different locations and analyzed. As evaluated by ATP, the viable microbial population was one to 3-logs higher than the aerobic plate counts. Three major clusters were found in relation to ATP content and identified with spores, bacteria, and yeast-fungi, consistent with their sizes and physiologies. Purified isolates representing these clusters were identified by 16S rDNA sequence analysis and their intracellular ATP concentrations measured as well. Gram-positive bacteria contain about 2-5 times more ATP per cell than Gram-negative species. Likewise, yeast and multi-cellular fungi have at least 100 times more ATP than the Gram-negative bacteria. However, spores exhibited only one-tenth of the ATP content of vegetative cells. It was interesting that 14 samples did not yield any cultivable microbes but the ATP assay successfully detected microbial contamination; this is attributed to the presence of viable but non-cultivable microorganisms.